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Assessment of Heavy Metal Levels in Various Species of Seaweeds in Misrata, Libya

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BSTRACT

Eight species of brown (*Phaeophyta*), red (*Rhodophyta*), and green (*Chlorophyta*) seaweed were collected from Misrata on the Libyan coast. The samples were digested using Milestone Start D Microwave. The levels of Cu, Cd, Pb, Ni, Zn, and Fe in the samples of the seaweed using an atomic absorption spectrophotometer. The higher levels of metals in seaweeds were Fe (588.1), Zn (164.28), Ni (16.67), Pb (8.136), Cu (4.857), and Cd (0.783) µg g^{-1} dry weight. Normal levels were found for Cd, Cu, Pb, Ni, Zn, and Fe. The results obtained show that the concentration of heavy metals decreased as follows: Cd< Cu< Pb< Ni< Zn < Fe.The results showed that brown seaweed recorded the highest accumulation of metals. Among the studied seaweeds, the highest concentration of metals was detected in *Padina pavonica*, and this species could be utilized for monitoring coastal waters on the Libyan coast.

الرصد البيئي للمعادن الثقيلة في الأعشاب البحرية من مصراتة، ليبيا

محمود عبدالمطلب باره، ازدهارعلى التلوتى، عائشه محمد المجدوب، فطيم التركى

جُمعت ثمانية أنواع من الطحالب البنية (*Phaeophyta*) والحمراء (*Rhodophyta*) والخضراء (*Chlorophyta*) من مصراتة على الساحل الليبي. خضعت العينات للهضم باستخدام جهاز الميكروويف Milestone Start D. حُددت مستويات النحاس (Cu) والكادميوم (Cd) والرصاص (Pb) والنيكل (Ni) والزنك (Zn) والحديد (Fe) في عينات الطحالب باستخدام مطياف الامتصاص الذري. سُجلت أعلى مستويات للمعادن في الطحالب، حيث بلغت 8.136 ميكروغرام/غرام من الوزن الجاف للرصاص، و0.783 ميكروغرام/غرام للكادميوم، و4.857 ميكروغرام/غرام للنحاس، و16.67 ميكروغرام/غرام للنيكل، و164.28 ميكروغرام/غرام للزنك، و588.1 ميكروغرام/غرام للحديد. بينما كانت المستويات طبيعية للكادميوم والنحاس والرصاص والنيكل والزنك والحديد. أظهرت النتائج أن تركيز المعادن الثقيلة انخفض بالترتيب التالي .Cd < Cu < Pb < Ni < Zn < Fe: كما أظهرت النتائج أن الطحالب البنية سجلت أعلى تراكم للمعادن. من بين الأعشاب البحرية التي تمت دراستها، تم الكشف عن أعلى تركيز للمعادن في نوع*Padina pavonica* ، ويمكن استخدام هذا النوع لمراقبة المياه الساحلية على الساحل الليبي.

INTRODUCTION

Heavy metals are among the dangerous pollutants that pose a significant threat to the environment. They are a source of concern for all countries of the world due to their toxicity and accumulation through the food chain, in addition to their negative effects on human health and the environment (Salah-Tantawy et al., 2022). Pollution from human activity in marine environments is a major pressure factor and brings attention to the systematic monitoring and management of pollutants such as heavy metals that effectively affect marine biota (Dadolahi-Sohrab et al., 2011). Trace concentrations of several hazardous and bioaccumulative contaminants are often present in water, and they are frequently discovered at increased levels in sediments. Risk evaluations that solely rely on information from water analysis studies might be deceptive. However, information derived from sediments may not accurately reflect the amounts of pollutants in the water column above it or provide insight into the patterns of contamination at higher trophic levels (Ali et al., 2021; Torres et al., 2008). The growing importance of heavy metals in environmental degradation, particularly in water bodies, is evident. While most research has focused on algae, macrophytes, and microalgae, these serve as useful indicators of their capacity to accumulate these metals (Guitouni et al., 2016). Around the world, macroalgae (seaweeds) have been widely employed to quantify heavy metal contamination in marine and freshwater ecosystems. Their capacity to accumulate metals to an acceptable degree, their lifespan, their prevalence at pollution places, and their ease of identification make them ideal as bioindicators (Al-Homaidan et al., 2011; Emin et al., 2019). Several studies have shown the various species of seaweed (macroalgae) uptake of heavy metals. Moreover, macroalgae (seaweeds) have progressively been employed as biodetectors to monitor xenobiotics in environments of marine. In the 1980s, the use of macroalgae (seaweed) in biomonitoring was very restricted, although many studies had shown that macroalgae (seaweed) are good bioindicators of seawater pollution with metals. However, the use of marine algae as experimental organisms in laboratory research on marine pollution has gradually increased (Wickramasinghe et al., 2017). The aim of the current paper is to assess the applicability of some highly widespread seaweeds growing in Misrata, Libya (Padina pavonica, Sargassum baccularia, Cystoseira sp., Dictyopteris, and Dilophus, which belong to class Phaeophyceae; Laurencia papillosa and Jania rubens, which belong to class Rhodophyta; and Dasycladus, which belongs to class Chlorophyta) to accumulate inorganic pollutants: Cd, Cu, Fe, Ni, Pb, and Zn. These species were selected for their abundance and biomonitoring capability.

MATERIALS AND METHODS

Seaweed samples were collected from the intertidal zone along the Libyan coast, specifically from the area near the city of Misrata, which is located about 200 km east of Tripoli **(Figure 1),** during 2006**.** The samples were gathered from depths ranging from 0 to 70 cm.

Fig. 1: Map depicting the locations within the study area.

Some types of seaweed are common on the Libyan Mediterranean coast.

Table 1: Presents three common types of algae found along the Libyan coast of the Mediterranean Sea.

Phaeophyta(Brown seaweed)	Rhodophyta(Red seaweed)	Chlorophyta(Green seaweed)
Sargassum baccularia	*Jania rubens*	*Dasycladus*
Padina pavonica	*Laurencia papillosa*	
Cystoseira sp.		
Dictyopteris		
Dilophus		

Sampling and Sample Preparation

The seaweed samples were cleaned by seawater at the sampling site and then transported to the laboratory on the same day in polyethylene bags kept at a temperature of -20°C. After arriving at the laboratory, they were carefully cleaned by seawater to eliminate sediment, particulate matter, and epifaunal and epiphytic types. Following that, they were washed with distilled water. The samples were dried overnight at 60°C in a Memmert oven, then homogenized by crushing each sample with a porcelain mortar and pestle. They were also kept away from metallic materials and dusty conditions to prevent pollution. The samples were digested using a Milestone Start D microwave.

Sample amount: 0.5 g

Reagents: 6 ml of nitric acid 65%, 2 ml of hydrogen peroxide 30%.

Microwave program

Step	Time	Temperature	Microwave power
1	10 min	200°C	Up to 1000 Watt*
2	20 min	200°C	Up to 1000 Watt*

Seaweed samples were diluted with distilled water to a final volume of 25 ml and analyzed.

Analysis by Atomic Absorption Spectroscopy (AAS)

The level of the metals was measured by the Solaar M Series AA Spectrometer (Thermo Scientific). Standard solutions were prepared from stock solutions (Fluka, Merck KGaA, Germany).

Statistical Analysis

The data represent the average and standard deviation (AVE ± SD) for triplicate samples. The results were analyzed using Microsoft Excel 2010, and statistical significance was determined for p-values ≤ 0.05 using ANOVA version 25.

.Metal pollution index (MPI)

To determine the total metal content in algal species within the area of study, the metal pollution index (MPI) was utilized **(**El-Din *et al*., 2014; Khaled *et al*., 2014).

$$\mathbf{MPI= (M_1 \times M_2 \times M_3 \times \ldots \times M_n)^{1/n}}$$

Where M is the concentration of metal and n is the metal number.

RESULTS AND DISCUSSION

Samples of seaweed collected at random intervals near the shoreline were analyzed to measure their metal content (cadmium, copper, lead, nickel, zinc, and iron). The results showed that seaweed collected from the Mediterranean coast can accumulate metals from its environment. The highest level was exhibited by Fe in all the species, irrespective of their classification; lower levels were recorded for Cd in all species. **Table 2** indicates the average level of heavy metals in seaweed. The overall order of accumulation is as follows: Fe > Zn > Ni > Pb > Cu > Cd. **Table 3** presents the maximum accumulation levels of various metals in different types of seaweed.

Table 2: Average concentration of seaweed groups (µg/g) dry weight.

seaweed	Cu	Cd	Pb	Ni	Zn	Fe
Brown (**Phaeophyta**)	3.316	0.461	5.688	12.62	96.47	344.2
Red (**Rhodophyta**)	2.551	0.390	3.892	9.14	62.07	149.5
Green (**Chlorophyta**)	2.093	0.267	3.332	8.29	48.45	110.2
P-value	0.026	0.020	0.025	0.049	0.088	0.001

Table 3 shows the highest levels of metals (µg/g) D.W in the different types of seaweed collected.

Seaweed species	Cu	Cd	Pb	Ni	Zn	Fe
Brown						
Sargassum baccularia	4.301	0.510	7.377	15.84	133.3	336.8
Padina pavonica	4.857	0.765	8.136	16.67	164.28	588.1
Cystoseira sp.	3.846	0.783	6.952	15.07	96.24	268.2
Dilophus	3.951	0.224	3.951	11.01	111.37	392.4
Dictyopteris	3.869	0.304	3.869	10.65	87.25	439.3
Red						
Jania rubens	3.195	0.523	5.442	9.485	102.15	200.1
Laurencia papillosa	3.076	0.424	4.618	10.27	89.24	187.7
Green						
Dasycladus	2.498	0.315	4.505	8.822	82.66	143.0

The results obtained show that the accumulation capacity of heavy metals was as follows: Phaeophyta < hodophyta hlorophyta.

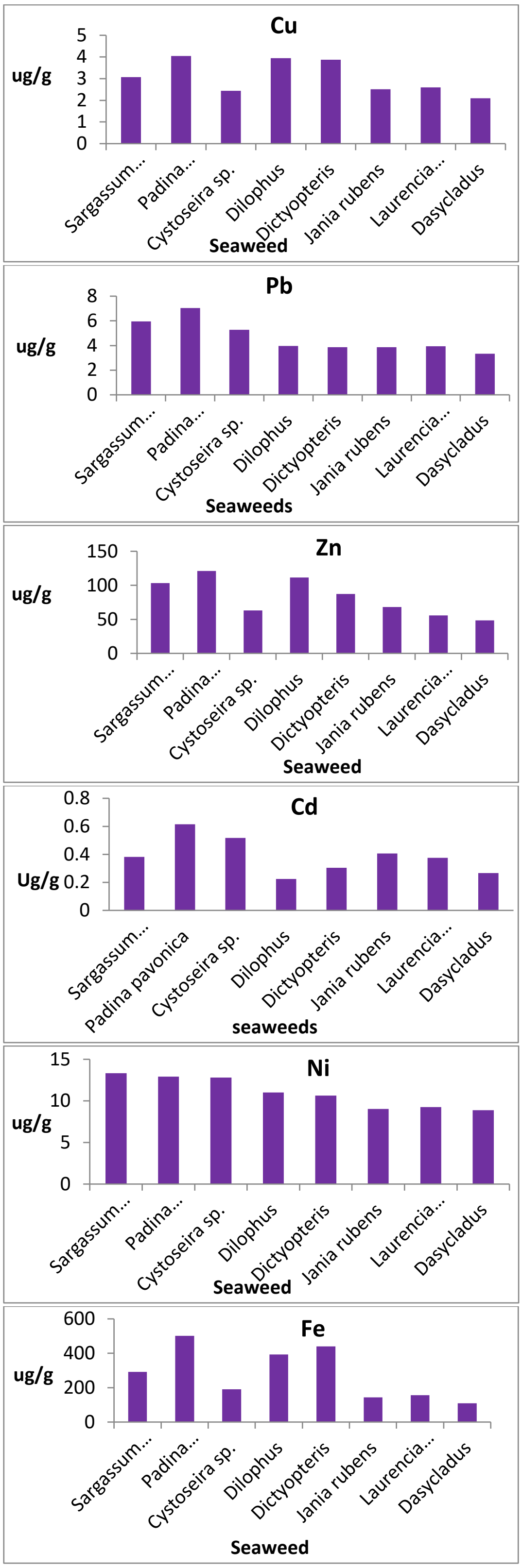

Fig.2. Levels of metals in seaweed species

During present research, the highest values of Cu (4.857), Cd (0.783), Pb (8.136), Ni (16.67), Zn (164.28), and Fe (588.1) $\mu g\ g^{-1}$ dry weight were observed in brown seaweed **(Table 2)**. Reports indicate that brown seaweed accumulates significant amounts of divalent metals. Ali *et al*., (2017), attributes this accumulation to the high levels of polysaccharides and binding polyphenols present in brown seaweed. The results indicated that the pattern of metal levels in seaweed samples decreased as follows: Fe > Zn > Ni > Pb > Cu > Cd. One-way ANOVA analysis indicates a significant difference in the level of metals among seaweed species ($p < 0.05$). The marine environment, including estuaries and coastal areas, is particularly vulnerable to exposure to non-essential metals like lead and cadmium. These metals often originate from agricultural waste that contains synthetic pesticides, as well as from household waste. Cadmium in seaweed > 2 $\mu g\ g^{-1}$ dry wt. has been coined for contaminated environments **(**Tayeb *et al*., 2015; Lozano *et al*., 2003). The European Commission (EC) Regulations No. 629/2008 (EC, 2008) and No. 488/2014 (EC, 2014) explicitly state the maximum permissible level of cadmium in seaweed, set at 3 ug/g**(** Paul *et al*.,2023). According to Strezov and Nonova, (2007**)**, this indicates the absence of dangerous human activities near the area. Cadmium concentration was higher in brown and red seaweed compared to green seaweed, which is consistent with a study by (Dadolahi-Sohrab *et al*. 2011**)**. In a study by Lee *et al*. (2022), slightly higher levels of cadmium were recorded in *Sargassum* seaweed compared with other marine seaweeds. Among brown seaweed species, *Sargassum* is considered an effective bioadsorbent for heavy metals, including cadmium, from contaminated wastewater. The levels of Pb recorded in *Padina pavonica* in this study were higher compared to other seaweeds (**Table 3**). The levels obtained for lead were < 10 ug/g dry weight, indicating no pollution of the area with this metal, according to Lozano *et al*., (2003). According to Anbazhagan *et al*. (2021), the mean level for Pb in brown seaweed (10.36 µg/g) is higher than the limits suggested by FSSAI, FAO/WHO, and CEVA. Levels of Cu range from the highest value of 4.857 in *Padina pavonica* to the minimum value of 2.498 $\mu g\ g^{-1}$ dw in *Dasycladales* (**Table 3**). During this study, among three classes of seaweeds, copper showed higher levels in Phaeophyta species compared to the others (**Table 2**). The presence of elevated copper levels presents a significant threat to all marine organisms. According to (Giusti, 2001; Caliceti *et al*., 2002; Al-Homaidan, 2007), copper pollution is associated with seaweed levels of > 20.0 $\mu g\ g^{-1}$ dry wt. According to Dahabi *et al*. (2023), the highest value obtained for copper in this study, 4.049 ug/g in *Padina pavonica* algae, is the lowest acceptable value from the IAEA, which is 23.2ug/g .During the present study, Ni levels decreased in various classes of seaweeds in the following order: Phaeophyta > Rhodophyta > Chlorophyta (**Table 2**). In this study, nickel reached its highest level at 16.67 µg/g. High levels of Ni were found in samples taken from polluted areas, ranging between 20 and 71 µg/g. This indicates the absence of oil pollution, industrial waste, and airborne contaminants in the area (Al-Homaidan, 2008). Zinc, an essential metal found in seaweed, provides several health benefits to humans (Bhuyan *et al*., 2024) .Average zinc levels were measured in eight species, with the highest value of 164.28 µg/g dw recorded in *Padina pavonica* (**Table 3**). Seaweed collected from an area polluted by a zinc and lead smelter (Sardinia, Italy) appeared to have high levels of zinc, reaching 780 ug/g dw (Trifan *et al*., 2015). However, statistically significant variance was observed between zinc values in the different species. In this study, Zn levels in various classes of seaweeds decreased as follows: Phaeophyta > Rhodophyta > Chlorophyta (**Table 2**). Fe is an important metal that occurs naturally in seaweed and provides several health advantages to humans (Bhuyan *et al*., 2024).Fe content was analyzed in eight species, and among all the minerals concentrated by the algae, Fe levels were found to be the highest, ranging from 143.0 to 588.1ug/g. Among various classes of seaweeds, Phaeophyta species appeared to have more Fe accumulated compared to others (**Table 2**). According to Al-Humaidan, (2007), the concentration of Fe in an area contaminated with Fe metal exceeds a concentration of 1000.0 µg/g. The accumulation of minerals depends on the type of polysaccharides present in seaweed, and since different elements have different electronegativity (a tendency to accept electrons), this is likely to affect the absorption of minerals in seaweed at different levels (Dadolahi-Sohrab *et al*. 2011; Rahhou *et al*., 2023).

Table 4. Metal Pollution Index (MPI) of heavy metals in seaweed species.

Seaweed	Cu	Cd	Pb	Ni	Zn	Fe	MPI
Sargassum Baccularia	3.065	0.381	5.947	13.34	103.38	292.5	11.76
Padina pavonica	4.049	0.616	7.038	12.91	121.12	500.9	15.31
Cystoseira sp.	2.440	0.517	5.265	12.82	63.03	191.5	9.95
Dilophus	3.951	0.224	3.951	11.01	111.37	392.4	10.80
Dictyopteris	3.869	0.304	3.869	10.65	87.25	439.3	10.98
Jania rubens	2.507	0.405	3.858	9.04	68.28	143.5	8.31
Laurencia papillosa	2.596	0.375	3.925	9.24	55.85	155.5	8.14
Dasycladus	2.094	0.267	3.333	8.88	48.46	110.2	6.62

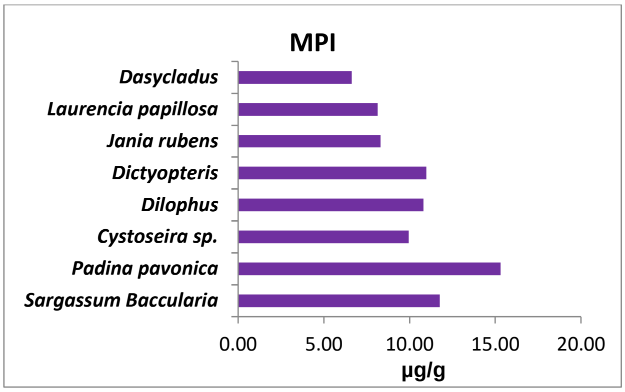

Fig3. Metal Pollution Index (MPI) of heavy metals in seaweed species.

Table. 5: MPI values in various classes of seaweed

classes of seaweed	Cu	Cd	Pb	Ni	Zn	Fe	MPI
Phaeophyta	3.316	0.461	5.688	12.62	96.47	344.2	12.3
Rhodophyta	2.551	0.39	3.892	9.14	62.07	149.5	8.2
Chlorophyta	2.093	0.267	3.332	8.29	48.45	110.2	6.5

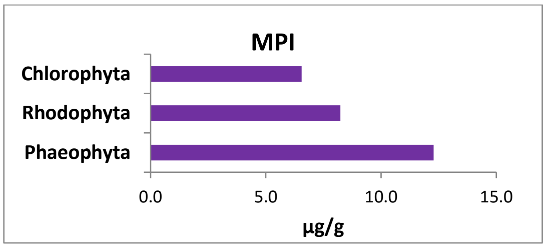

Fig. 4: MPI values in different classes of seaweed

Seaweed	Cd	Pb	MPI(Cd+Pb)
Padina pavonica	0.616	7.038	2.08
Jania rubens	0.405	3.858	1.25
Dasycladus	0.267	3.333	0.943

Table 6. Metal Pollution Index (MPI) of (Cd+Pb) in seaweed species.

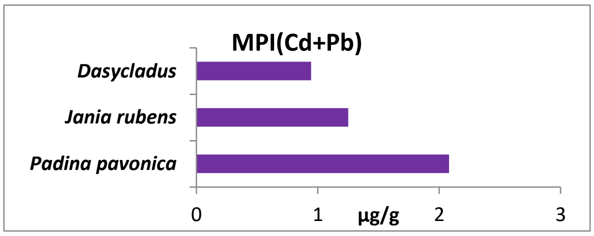

Fig. 5: Metal Pollution Index (MPI) of (Cd,Pb) in seaweed species.

The MPI values of various classes showed the highest and lowest in Phaeophyta and Chlorophyta, respectively (Table 5, Fig. 4). Meanwhile, species MPI values were decreased in the following order: *Padina pavonica* > *Sargassum bacularia* > *Dictyopteris* > *Dilophus* > *Cystoseira sp.* > *Jania Rubens* > *Laurencia papillosa*, and *Dasycladus* (Table 4, Fig 3). Higher MPI indicated more metal uptake by seaweeds in this area (Dadolahi-Sohrab *et al.*, 2011). According to El-Nimr *et al.*, (2012), the study of partially and totally MPI reveals that the metal content depends on the seaweed species rather than on the class of seaweed. *Padina pavonica*, a type of brown seaweed, recorded the highest (MPI) Cd+Pb due to its calcareous structure. Brown seaweeds have a high capability to remove heavy metals. Brown seaweeds are superior to other types of seaweeds, such as red and green seaweed, in terms of biosorption capacity (Tsui *et al.*, 2006). The greater ability of macroalgae to accumulate heavy metals is believed to be linked to their simple cell structure, which may make them more resistant to elevated levels of heavy metals compared to higher animals (Tresnati et al., 2021). According to Kim et al. (2003), the greater levels of heavy metals were accumulated in brown seaweeds.

CONCLUSION

Based on the findings obtained in the current study, the following can be concluded: Brown seaweeds are suitable biomonitors of heavy metal contamination on the Libyan coast. Because brown seaweed has the highest amount of polysaccharides, this seaweed is considered suitable and excellent for binding heavy metals (Murphy, 2007). *Padina pavonica* and *Sargassum bacularia* are probably the best species for monitoring metal pollution because of their high accumulation of metals. The levels of Cu, Cd, Pb, Ni, Zn, and Fe were similar to those obtained from unpolluted areas. Seaweed has proven to be of great importance to researchers due to its high capacity for accumulating minerals, its low cost, its renewable nature, and its availability

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